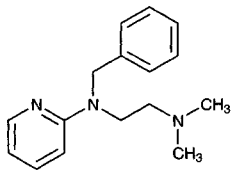
KEY WORDS
serum; pharmacokinetics

REFERENCE
Stolk,L.; Siddiqui,A.H.; Cormane,R.H. Serum levels of trimethylpsoralen after oral administration, *Br.J.Dermatol.*, **1981**, *104*, 443–445.

Tripelennamine



Molecular formula: $C_{16}H_{21}N_3$
Molecular weight: 255.36
CAS Registry No.: 91-81-6, 6138-56-3 (citrate), 154-69-8 (HCl)
Merck Index: 9868
Lednicer No.: 1 51

SAMPLE
Matrix: blood, milk
Sample preparation: Centrifuge milk at 1200 g, remove the middle aqueous layer. 1 mL Plasma or milk + 50 μL MeOH:water 50:50 + 50 μL 4 μg/mL protriptyline in MeOH:water 50:50, mix, inject a 250 μL aliquot of this mixture on to column A and elute to waste with mobile phase A. After 3 min backflush the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 37-50 μm 10 × 1.5 Corasil RP C18; B 100 × 4 Techsphere 3CN (HPLC Technology)
Mobile phase: A water; B MeCN:50 mM pH 7.2 acetate buffer 70:30
Flow rate: A 0.8; B 0.9
Injection volume: 250
Detector: UV 246

CHROMATOGRAM
Retention time: 5.2
Internal standard: protriptyline (6.8)
Limit of detection: 2 ng/mL

KEY WORDS
column-switching; cow; plasma

REFERENCE
Dadgar,D.; Power,A. Applications of column-switching techniques in biopharmaceutical analysis. II. High-performance liquid chromatographic determination of tripelennamine in bovine plasma and milk, *J.Chromatogr.*, **1987**, *421*, 216–222.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 μm) (discard first 10 mL of filtrate), inject a 20 μL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeOH:3 mM ammonium acetate 90:10
Flow rate: 1.3
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.9

OTHER SUBSTANCES
Also analyzed: chlorpheniramine, cyclizine, doxylamine, mesoridazine, pentazocine, promethazine, protriptyline, pyrilamine, pyrimethamine

KEY WORDS
tablets; syrups; elixirs; injections

REFERENCE
Walker,S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 539–542.

SAMPLE
Matrix: fungal incubations
Sample preparation: 40 mL Fungal incubation + 100 mL water, centrifuge, wash pellet with 50 mL MeOH. Combine the supernatant and the MeOH wash and extract three times with 150 mL portions of dichloromethane, filter the extracts through a plug of anhydrous sodium sulfate, evapporate the filtrate to dryness under reduced pressure at 50°, reconstitute with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere cyano
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM KH_2PO_4 containing 20 mM trimethylamine, pH 7.0.)
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 17.9

OTHER SUBSTANCES
Extracted: metabolites, methapyrilene, thenyldiamine

REFERENCE
Cerniglia,C.E.; Hansen,E.B.,Jr.; Lambert,K.J.; Korfmacher,W.A.; Miller,D.W. Fungal transformations of antihistamines: metabolism of methapyrilene, thenyldiamine and tripelennamine to *N*-oxide and *N*-demethylated derivatives, *Xenobiotica*, **1988**, *18*, 301–312.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.1

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine,

buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, triprolidine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: thonzylamine, pheniramine, chlorpheniramine, brompheniramine, phenindamine, phenyltoxamine, clemizole

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REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 0.5 mg/mL solution in water, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM

Retention time: 7.3

OTHER SUBSTANCES

Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate
Interfering: tranylcypromine

KEY WORDS

details for purification of triethylamine in paper

REFERENCE

Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isox-

suprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac 201HS54 C18
Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min
Flow rate: 1.5
Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, triprolidine, cyclizine, methaphenilene, pyrrobutamine, meclizine, buclizine

REFERENCE

Vydac HPLC Catalog, 1994-5.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 7.71

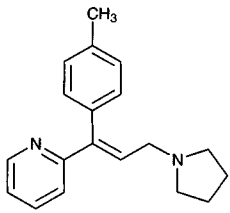
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Triprolidine



Molecular formula: $C_{19}H_{22}N_2$
Molecular weight: 278.40
CAS Registry No.: 468-12-4, 6138-79-0 (HCl monohydrate), 550-70-9 (HCl)
Merck Index: 9877
Lednicer No.: 1 78

SAMPLE

Matrix: blood
Sample preparation: 1 mL Serum + 250 µL 10% KOH + 5 mL ether, vortex, centrifuge. Remove ether layer and add it to 100 µL 0.5% phosphoric acid, vortex, centrifuge, remove most of ether layer and discard it, remove traces of ether by nitrogen at room temperature for 2-3 min, inject all of aqueous layer.

HPLC VARIABLES

Column: Waters CN reverse-phase radial compression
Mobile phase: MeCN:buffer 27:73 (Buffer was 75 mM pH 3.0 phosphate buffer containing 20 mM dibutylamine and 50 ng/mL triprolidine.)
Flow rate: 1
Injection volume: 100
Detector: UV 229

CHROMATOGRAM

Retention time: 3.6
Internal standard: triprolidine

OTHER SUBSTANCES

Simultaneous: hydroxyzine

KEY WORDS

serum; triprolidine is IS

REFERENCE

Simons,F.E.R.; Simons,K.J.; Frith,E.M. The pharmacokinetics and antihistaminic of the H_1 receptor antagonist hydroxyzine, *J.Allerg.Clin.Immunol.*, **1984**, *73*, 69–75.

SAMPLE

Matrix: blood
Sample preparation: Condition a 200 mg Bond Elut C8 SPE cartridge with 4 mL MeOH and 4 mL 100 mM pH 5 ammonium acetate. 1 mL Plasma + 1 mL 100 mM HCl, add to the SPE cartridge, wash with 2 mL MeOH:water 30:70, elute with 4 mL MeOH:1 M HCl in MeOH 97:3. Evaporate the eluate to dryness under a stream of nitrogen with gentle heating, reconstitute the residue in 200 µL mobile phase, filter (0.45 µm), inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm Spherisorb C18
Column: two 150 × 4.5 5 μm Econosphere C8 columns in series
Mobile phase: MeCN:buffer 22:78 (Buffer was 100 mM ammonium acetate containing 6 mM n-heptylamine, adjusted to pH 2.5 with perchloric acid).
Flow rate: 0.9
Detector: F ex 300 em 490

CHROMATOGRAM
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites (UV 290)

KEY WORDS
dog; plasma; SPE; pharmacokinetics

REFERENCE
McNulty,M.J.; Deal,D.L.; Page,T.L.; Chandrasurin,P.; Findlay,J.W.A. Disposition of triprolidine in the male beagle dog, *Drug Metab.Dispos.*, **1992**, *20*, 928–935.

SAMPLE
Matrix: blood
Sample preparation: 600 μL Serum + 100 μL 400 mM NaOH + 7 mL ethyl acetate, vortex for 1 min, centrifuge at 1000 g for 10 min. Remove the organic layer and add it to 150 μL 100 mM HCl, vortex for 1 min, centrifuge, inject a 50 μL aliquot of the aqueous phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb-C6
Mobile phase: MeCN:30 mM KH_2PO_4 45:55 containing 2 g sodium hexanesulfonate, pH adjusted to 2.7 with phosphoric acid
Column temperature: 30
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: triprolidine

OTHER SUBSTANCES
Extracted: clozapine

KEY WORDS
serum; triprolidine is IS

REFERENCE
Volpicelli,S.A.; Centorrino,F.; Puopolo,P.R.; Kando,J.; Frankenburg,F.R.; Baldessarini,R.J.; Flood,J.G. Determination of clozapine, norclozapine, and clozapine-N-oxide in serum by liquid chromatography, *Clin.Chem.*, **1993**, *39*, 1656–1659.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 5.34
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, CSF
Sample preparation: Plasma. Centrifuge blood at 7000 rpm, decant 100 μL plasma. Mix 100 μL plasma with 200 μL acetone, centrifuge at 7000 rpm for 5 min. Evaporate the supernatant under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot. CSF. Add 25 μL water to 25 μL CSF, mix with 50 μL acetone, centrifuge at 7000 rpm for 5 min, decant the supernatant, evaporate under a stream of nitrogen, reconstitute the residue with mobile phase, inject an aliquot.

HPLC VARIABLES

Column: Rainin C18

Mobile phase: MeOH:water 61.9:38.1 containing 0.2% diethylamine

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Chou,K.-J.; Donovan,M.D. Distribution of antihistamines into the CSF following intranasal delivery, *Biopharm.Drug Dispos.*, **1997**, *18*, 335–346.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
Column: 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
Mobile phase: MeCN:100 mM NaH_2PO_4:diethylamine 40:57.5:2.5
Flow rate: 2
Injection volume: 30
Detector: UV 220

CHROMATOGRAM
Retention time: 16.05
Internal standard: cianopramine (8.93)

OTHER SUBSTANCES
Extracted: amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine
Noninterfering: dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

KEY WORDS
serum; whole blood; liver

REFERENCE
McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.133

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: feces, urine
Sample preparation: Urine. Condition a 200 mg Bond Elut C8 SPE cartridge with 4 mL MeOH and 4 mL 100 mM pH 5 ammonium acetate. Dilute urine five-fold with 100 mM pH 5 ammonium acetate, add to the SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH:1 M HCl in MeOH 97:3. Evaporate the eluate to dryness under a stream of nitrogen with gentle heating, reconstitute the residue in 200 μL MeCN:100 mM ammonium acetate 5:95, vortex, sonicate, centrifuge, filter (0.45 μm), inject an aliquot. Feces. Lyophilize, grind to a powder, add 100-200 mg to 2 mL 2% ammonium hydroxide, heat at 37° for 30-60 min, add 8 mL MeOH, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen with gentle heating, reconstitute the residue in 300 μL MeOH:water 50:50, centrifuge, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Guard column: 5 μm Spherisorb C18
Column: two 150 × 4.5 5 μm Econosphere C8 columns in series
Mobile phase: Gradient. A was MeCN:buffer 10:90 (buffer was 100 mM ammonium acetate containing 10 mM n-heptylamine, adjusted to pH 2.5 with perchloric acid). B was MeCN:buffer 20:80 (buffer was 100 mM ammonium acetate containing 15 mM n-heptylamine, adjusted to pH 2.5 with perchloric acid). Urine: A:B 100:0 for 10 min, to 0:100 over 15 min. Feces: A:B 100:0 for 20 min, to 0:100 over 20 min.
Flow rate: 0.9
Detector: UV 290 or radioactivity

CHROMATOGRAM
Retention time: 36 (urine), 43 (feces)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
dog; SPE; pharmacokinetics

REFERENCE
McNulty,M.J.; Deal,D.L.; Page,T.L.; Chandrasurin,P.; Findlay,J.W.A. Disposition of triprolidine in the male beagle dog, *Drug Metab.Dispos.*, **1992**, *20*, 928–935.

SAMPLE
Matrix: fungal incubations
Sample preparation: Extract fungal incubation with dichloromethane, evaporate the extract to dryness, reconstitute with 5 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: cyano (Beckman/Altex)
Mobile phase: MeCN:buffer 35:65 (Buffer was 80 mM ammonium acetate containing 10 mM tripropylamine, adjusted to pH 6.5 with trifluoroacetic acid.)
Flow rate: 1.25
Injection volume: 20
Detector: MS, Delsi-Nermag R1010C quadrupole, Vestec thermospray interface, discharge-off, filament off, thermospray source block 268°, control temperature 101°, tip 217°, m/z 279

CHROMATOGRAM
Retention time: 19
Limit of detection: 10 μg

OTHER SUBSTANCES
Extracted: metabolites

REFERENCE
Hansen,E.B.J.; Getek,T.A.; Korfmacher,W.A. Application of HPLC-thermospray ionization mass spectrometry for the analysis of triprolidine and its metabolite hydroxymethyltriprolidine in biological samples, *J.Anal.Toxicol.*, **1989**, *13*, 185–187.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 3.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metopro-

lol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48

Flow rate: 1.5

Injection volume: 10

Detector: UV

CHROMATOGRAM

Retention time: k' 2.40

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 7.6 μg/mL solution, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: Supelguard LC-8-DB (Supelco)

Column: 50 × 4.6 Supelcosil LC-8-DB

Mobile phase: MeCN:buffer 10:90 containing 0.02% triethylamine (Buffer was KH_2PO_4 adjusted to pH 2.0 with phosphoric acid.)

Column temperature: 35

Flow rate: 2

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Simultaneous: chlorpheniramine, methscopolamine, phenylpropanolamine, pseudoephedrine

REFERENCE
Supelco Catalog, **1992**, p. 179.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Supelguard (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeCN:MeOH:buffer 19:28:53 (Buffer was 50 mM KH_2PO_4 containing 0.2% triethylamine, pH 2.5.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: chlorcyclizine, chlorpheniramine, clonidine, diphenhydramine, promethazine, pyrilamine

REFERENCE
Supelco Catalog, **1994**, p. 768.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, meth-

apyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Vydac 201HS54 C18
Mobile phase: Gradient MeCN:25 mM pH 3.6 phosphate buffer from 20:80 to 70:30 over 20 min
Flow rate: 1.5
Detector: UV 220 (from Vydac Applications Brochure)

CHROMATOGRAM

Retention time: 8.6

OTHER SUBSTANCES

Simultaneous: chlorcyclizine, tripelennamine, cyclizine, methaphenilene, pyrrobutamine, meclizine, buclizine

REFERENCE

Vydac HPLC Catalog, 1994-5.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 12.13

OTHER SUBSTANCES

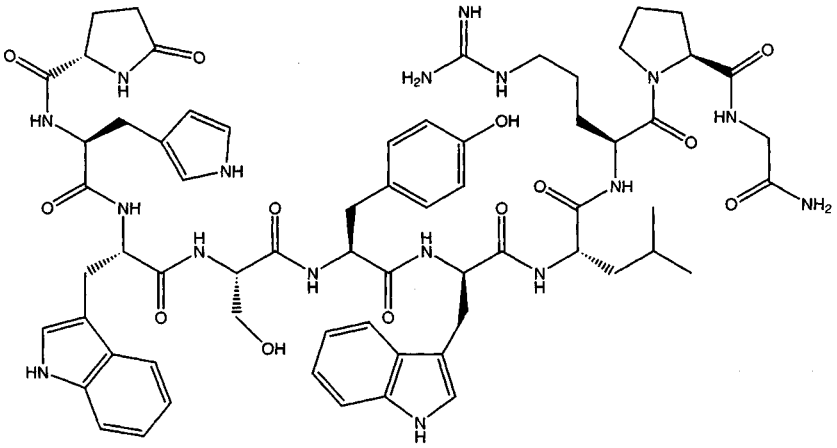
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Triptorelin

Molecular formula: $C_{64}H_{82}N_{18}O_{13}$
Molecular weight: 1311.47
CAS Registry No.: 57773-63-4, 140194-24-7 (acetate)
Merck Index: 9878



SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 4 5 μm Lichrosphere 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 22:78
Flow rate: 1
Detector: UV 214

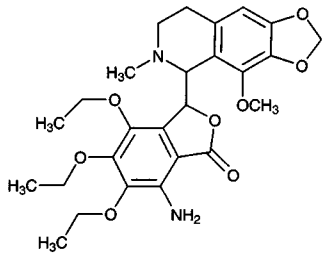
OTHER SUBSTANCES

Simultaneous: degradation products
Also analyzed: goserelin

REFERENCE

Hoitink,M.A.; Beijnen,J.H.; Boschma,M.U.S.; Bult,A.; Hop,E.; Nijholt,J.; Versluis,C.; Wiese,G.; Underberg,W.J.M. Identification of the degradation products of gonadorelin and three analogues in aqueous solution, *Anal.Chem.*, **1997**, *69*, 4972–4978.

Tritoqualine



Molecular formula: $C_{26}H_{32}N_2O_8$
Molecular weight: 500.55
CAS Registry No.: 14504-73-5
Merck Index: 9894

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 214.6

CHROMATOGRAM

Retention time: 20.968

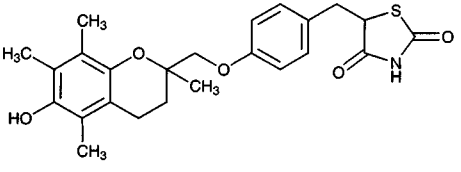
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Troglitazone



Molecular formula: $C_{24}H_{27}NO_5S$
Molecular weight: 441.55
CAS Registry No.: 97322-87-7
Merck Index: 9898

SAMPLE

Matrix: blood
Sample preparation: Add 100 µL 5 µg/mL IS and 500 µL PIC A (Waters) to 500 µL plasma add 5 mL ethyl acetate:hexane 90:10. Shake for 20 min, centrifuge at 1000 g for 5 min. Evap-

orate the organic layer to dryness under a gentle stream of nitrogen at 40°. Reconstitute the residue in 500 μL MeCN:water 50:50. Inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 6.0 5 μm ODS (YMC, USA)
Mobile phase: MeCN:water:phosphoric acid 60:40:0.08
Flow rate: 1.2
Injection volume: 50
Detector: UV 230

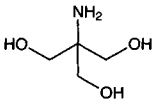
CHROMATOGRAM
Retention time: 23.2
Internal standard: 9-acetylanthracene (27.2)
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Loi,C.-M.; Randinitis,E.J.; Vassos,A.B.; Kazierad,D.J.; Koup,J.R.; Sedman,A.J. Lack of effect of type II diabetes on the pharmacokinetics of troglitazone in a multiple-dose study, *J.Clin.Pharmacol.*, **1997**, *37*, 1114–1120.

Tromethamine



Molecular formula: $C_4H_{11}NO_3$
Molecular weight: 121.14
CAS Registry No.: 77-86-1
Merck Index: 9902

SAMPLE
Matrix: amniotic fluid, blood, CSF, urine
Sample preparation: Plasma. Condition a 100 mg Bond Elut SCX (propylbenzenesulfonic acid, H^+ form) SPE cartridge with 1 mL 50 mM HCl, 1 mL MeOH, 2 mL water, and 1 mL 50 mM HCl. 100 μL Plasma + 100 μL 250 μM norleucine in 100 mM HCl + 10 mg solid sulfosalicylic acid + 800 μL acetone or MeOH, mix, centrifuge, add a 50 μL aliquot to the SPE cartridge, wash with 2 mL water, elute with two 500 μL portions of MeOH:water:triethylamine 40:40:20, dry the eluate under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Dried blood. Add 25 μL 250 μM norleucine in 100 mM HCl to a 6 mm filter paper disc containing dried blood, add 100 μL MeCN, let stand for 30 min, centrifuge, remove a 75 μL aliquot of the supernatant, evaporate to dryness under reduced pressure, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 2 min, evaporate to dryness under vacuum, reconstitute with 50 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Amniotic fluid, CSF. Mix amniotic fluid or CSF with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a 50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 50 (CSF) or 100 (amniotic fluid) μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot. Urine. Dilute urine with water to a creatinine concentration of 1 mM, mix an aliquot with an equal volume of 250 μM norleucine in 100 mM HCl, filter (Centrifree 10000 MW cutoff) while centrifuging at 2200 g. Evaporate a

50 μL aliquot of the ultrafiltrate to dryness under vacuum, add 10 μL MeOH:1 M sodium acetate:triethylamine 40:40:20, dry under vacuum at 70 mTorr, reconstitute with 20 μL MeOH:triethylamine:water:phenylisothiocyanate 70:10:10:10, let stand at room temperature for 20 min, evaporate to dryness under vacuum, reconstitute with 100 μL MeCN:5 mM pH 7.4 sodium phosphate buffer 5:95, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 Pico-Tag amino acid column (Waters)
Mobile phase: Gradient. A was MeCN:70 mM pH 6.55 sodium acetate 2.5:97.5. B was MeCN:MeOH:water 45:15:40. A:B 100:0 for 13.5 min, to 97:3 (step gradient), to 94:6 over 10.5 min (Waters curve 8 (slightly concave)), to 91:9 over 6 min (Waters curve 5 (slightly convex)), to 66:34 over 20 min, maintain at 66:34 for 12 min, to 0:100 over 0.5 min, maintain at 0:100 for 4 min, return to initial conditions over 0.5 min.
Column temperature: 46
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 34.47
Internal standard: norleucine (55.07)

OTHER SUBSTANCES
Extracted: α-alanine, alanine, alloisoleucine, β-aminoadipic acid, 4-aminobenzoic acid, gamma-aminobutyric acid, β-amino-n-butyric acid, gamma-amino-n-butyric acid, 4-aminohippuric acid, β-aminoisobutyric acid, 4-aminophenylacetic acid, α-aminophenylacetic acid, 3-amino-3-phenylpropionic acid, δ-amino-n-valeric acid, ammonia, anserine, arginine, asparagine, aspartic acid, aspartylglucosamine, carnosine, citrulline, cystathionine, cysteic acid, cysteine, cysteine-homocysteine (mixed disulfide), cystine, ethanolamine, ethionine, ethylamine, galactosamine, glucosamine, glutamic acid, glutamine, glutathionine (oxidized), glycine, glycylglycine, glycylhistidine, glycylleucine, glycylphenylalanine, glycyltyrosine, histidine, homoarginine, homocitrulline, homoserine, homocystine, 3-hydroxyanthranilic acid, 3-hydroxykynurenine, hydroxyproline, isoleucine, kynurenine, leucine, levodopa, lysine, methionine sulfone, methionine, 3-methylhistidine, 1-methylhistidine, ornithine, phenylalanine, phosphoethanolamine, phosphoserine, proline, sarcosine, serine, serotonin, taurine, threonine, tryptophan, tyrosine, valine
Noninterfering: cadaverine, 2-phenylethylamine

KEY WORDS
derivatization; SPE; ultrafiltrate; plasma; dried blood

REFERENCE
Davey,J.F.; Ersser,R.S. Amino acid analysis of physiological fluids by high-performance liquid chromatography with phenylisothiocyanate derivatization and comparison with ion-exchange chromatography, *J.Chromatogr.*, **1990**, *528*, 9–23.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 50 μL 5.027 mg/mL 2,3-butanediol in water + 1 mL 4 M NaOH + 200 μL benzoyl chloride, rotate for 10 min, add 2 drops 13.1 mg/mL sodium glycine salt in water, let stand for 2-3 min, add 8 mL hexane, rotate for 5 min, centrifuge. Remove the upper organic layer and evaporate it to dryness, reconstitute the residue in 300 μL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Column: reverse-phase 10 C ODS
Mobile phase: MeCN:MeOH water 25:50:25
Flow rate: 2
Injection volume: 50
Detector: UV 237

CHROMATOGRAM
Retention time: 4

Internal standard: 2,3-butanediol (3)
Limit of detection: 20 μg/mL

KEY WORDS
serum; derivatization

REFERENCE
Blanke,S.R.; Blanke,R.V. The Schotten-Baumann reaction as an aid to the analysis of polar compounds: application to the determination of tris(hydroxymethyl)aminomethane (THAM), *J.Anal.Toxicol.*, **1984**, *8*, 231–233.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 50 μL 750 μg/mL 2,3-butanediol in water + 200 μL 4 M NaOH, vortex briefly, add 40 μL benzoyl chloride, vortex for 3 min, add 8 mL MTBE:MeOH 99:1, vortex for 3 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness in a vortex evaporator at 55° for 30 min, cool to room temperature, reconstitute the residue in 500 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Ultrasphere octyl
Mobile phase: Gradient. MeCN:25 mM pH 6.5 potassium phosphate buffer 40:60 45:55 for 10 min, to 73:27 over 7 min (concave gradient), return to initial conditions over 1 min.
Flow rate: 3
Injection volume: 10
Detector: UV 237

CHROMATOGRAM
Retention time: 15.0
Internal standard: 2,3-butanediol (7.1)
Limit of detection: 282 ng/mL

KEY WORDS
plasma; derivatization

REFERENCE
Gumbhir,K.; Mason,W.D. High-performance liquid chromatographic method for the determination of tris(hydroxymethyl)aminomethane (tromethamine) in human plasma, *J.Chromatogr.*, **1992**, *583*, 99–104.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 100 μL Plasma + 100 μL 400 μg/mL 2-amino-2-ethyl-1,3-propanediol + 100 μL 7% perchloric acid, vortex, centrifuge at 2000 g for 5 min. Remove a 200 μL aliquot of the supernatant and add it to 300 μL 200 mM pH 9.2 borate buffer, vortex briefly, add 40 μL 4 mg/mL 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole in MeCN, vortex briefly, heat at 80° for 30 min, cool to room temperature in a water bath for 10 min, add 500 μL 5 M NaOH, vortex for 10 s, add 100 μL benzoyl chloride, vortex for 1 min, add 2 mL ethyl acetate:MeOH 90:10, rotate for 10 min, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under vacuum for 1 h, reconstitute the residue in 1 mL MeCN:10 mM phosphoric acid 80:20, inject a 50 μL aliquot. Urine. 200 μL Urine + 100 μL 200 μg/mL 2-amino-2-ethyl-1,3-propanediol + 100 μL water + 100 μL 10 mM pH 8.5 borate buffer + 200 μL 4 mg/mL 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole in MeCN, vortex briefly, heat at 80° for 30 min, cool to room temperature in a water bath for 10 min, add 500 μL 5 M NaOH, vortex for 10 s, add 100 μL benzoyl chloride, vortex for 1 min, add 2 mL ethyl acetate:MeOH 90:10, rotate for 10 min, centrifuge at 2000 g for 5 min. Remove the supernatant and evaporate it to dryness under vacuum for 1 h, reconstitute the residue in 1 mL MeCN:10 mM phosphoric acid 80:20, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 Supelcosil octadecylsilane silica column
Column: 250 × 4.6 Supelcosil octadecylsilane
Mobile phase: MeCN:10 mM phosphoric acid 70:30, adjusted to pH 2.5 with 10 M KOH

Flow rate: 1
Injection volume: 50
Detector: F ex 460 em 520

CHROMATOGRAM
Retention time: 12
Internal standard: 2-amino-2-ethyl-1,3-propanediol (10)
Limit of quantitation: 5 μg/mL (urine), 1 μg/mL (plasma)

KEY WORDS
plasma; derivatization

REFERENCE
Morris,M.J.; Hsieh,J.Y.-K. Determination of tris(hydroxymethyl)aminomethane (tromethamine) in human plasma and urine by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1993**, *622*, 87–92.

SAMPLE
Matrix: formulations
Sample preparation: Dilute formulation with water to obtain a tromethamine concentration of 260 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 IonPac CS5 (Dionex)
Mobile phase: 10 mM HCl (If necessary regenerate column with MeCN:water 5:95 at 1 mL/min for 30 min and with 1 M HCl at 1 mL/min for 1 h.)
Flow rate: 1.5
Injection volume: 20
Detector: Conductivity

CHROMATOGRAM
Retention time: 5.4

OTHER SUBSTANCES
Simultaneous: sodium
Noninterfering: lodoxamide

REFERENCE
Hall,R.E.; Havner,G.D.; Good,R.; Dunn,D.L. Ion chromatographic method for rapid and quantitative determination of tromethamine, *J.Chromatogr.A*, **1995**, *718*, 305–308.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 20 × 4 5 μm Supelcosil LC-18
Column: 250 × 4 5 μm Supelcosil LC-18
Mobile phase: 5 mM pH 6.0 acetate buffer
Flow rate: 1.2
Detector: UV 270

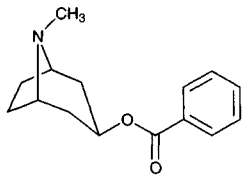
CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: guanine, xanthine

KEY WORDS
tromethamine is from Tris buffer

REFERENCE
Canepari,S.; Castellano,P.; Cernia,E.; Girelli,A.M.; Bozza,A. Determination of guanase activity in normal and pathological sera by high-performance liquid chromatography, *Biomed.Chromatogr.*, **1995**, *9*, 130–134.

Tropacocaine



Molecular formula: $C_{15}H_{19}NO_2$
Molecular weight: 245.32
CAS Registry No.: 537-26-8
Merck Index: 9904

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

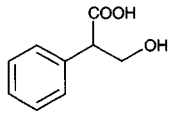
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine,

quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Tropic acid



Molecular formula: $C_9H_{10}O_3$
Molecular weight: 166.18
CAS Registry No.: 529-64-6
Merck Index: 9910

SAMPLE

Matrix: bulk
Sample preparation: Add a sample to 60 mg soy lecithin, make up to 50 mL with 0.1 mM HCl, sonicate, filter through a 0.2 μm nylon filter, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: Gradient. A was MeCN:buffer 20:80. B was MeCN:buffer 45:55. A:B 100:0 for 5 min, from 100:0 to 0:100 in 3 min, maintain at 0:100 for 4 min, from 0:100 to 100:0 in 1 min, maintain at 100:0 for 6 min. (Buffer was 100 mM KH_2PO_4 prepared by dissolving 27.2 g KH_2PO_4 in water, adjusting pH to 4.0 with 85% phosphoric acid and making up to 2 L with water.)
Column temperature: 35
Flow rate: 1 for 12 min, 2 for 7 min
Injection volume: 20
Detector: UV 210

CHROMATOGRAM

Retention time: 5.96

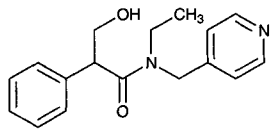
OTHER SUBSTANCES

Simultaneous: ipratropium bromide, N-isopropyl-nor-atropine, 8-s-ipratropium bromide, apoipratropium bromide

REFERENCE

Simms,P.J.; Towne,R.W.; Gross,C.S.; Miller,R.E. The separation of ipratropium bromide and its related compounds, *J.Pharm.Biomed.Anal.*, **1998**, *17*, 841–849.

Tropicamide



Molecular formula: $C_{17}H_{20}N_2O_2$
Molecular weight: 284.36
CAS Registry No.: 1508-75-4
Merck Index: 9911

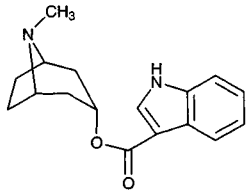
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3022 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 254

KEY WORDS
chiral; α = 1.10 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Tropisetron



Molecular formula: $C_{17}H_{20}N_2O_2$
Molecular weight: 284.36
CAS Registry No.: 89565-68-4
Merck Index: 9914

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma and 500 µL 50 mM NaOH, extract with diethyl ether. Remove the organic layer and add it to 200 µL 20 mM HCl, extract. Remove the aqueous layer and add it to 50 µL 1.5% triethylamine, inject a 150 µL aliquot.

HPLC VARIABLES
Column: Spherisorb RP8
Mobile phase: MeCN:0.3% triethylamine 65:35
Injection volume: 150
Detector: UV 283

CHROMATOGRAM
Limit of quantitation: 0.3 ng/mL

KEY WORDS
plasma

REFERENCE
Fischer,V.; Baldeck,J.-P.; Tse,F.L.S. Pharmacokinetics and metabolism of the 5-hydroxytryptamine antagonist tropisetron after single oral doses in humans, *Drug Metab.Dispos.*, **1992**, *20*, 603–607.

SAMPLE
Matrix: feces
Sample preparation: Exhaustively extract feces with MeOH, inject an aliquot of the extract.

HPLC VARIABLES
Column: 250 × 7 7 µm Lichrosorb RP18
Mobile phase: Gradient. A was 0.01% triethylamine adjusted to pH 8.4 with 330 mM phosphoric acid. B was MeCN. A:B 100:0 for 2 min, to 92:8 over 1 min, to 80:20 over 27 min, to 0:100 over 20 min.

Detector: UV 280 or radioactivity

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics

REFERENCE
Fischer,V.; Baldeck,J.-P.; Tse,F.L.S. Pharmacokinetics and metabolism of the 5-hydroxytryptamine antagonist tropisetron after single oral doses in humans, *Drug Metab.Dispos.*, **1992**, *20*, 603–607.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4 Nucleosil C18
Mobile phase: MeOH:THF:buffer 30:5:65 (Buffer was 100 mM triethylamine adjusted to pH 3.0 with nitric acid.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 10.10

OTHER SUBSTANCES
Simultaneous: granisetron, ondansetron

REFERENCE
Barbato,F.; Immacolata La Rotonda,M.; Quaglia,F. Retention behaviour of anti-emetic serotonin antagonists in reversed phase high performance liquid chromatography, *Farmaco*, **1995**, *50*, 875–880.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine at 3000 rpm for 15 min, inject an aliquot

HPLC VARIABLES
Column: 250 × 7 7 μm Lichrosorb RP18
Mobile phase: Gradient. MeCN:0.3% triethylamine (?) from 5:95 to 15:85 over 30 min, to 20:80 over 10 min, to 90:10 over 20 min
Flow rate: 3.1
Detector: UV 280 or radioactivity

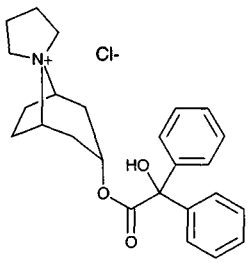
CHROMATOGRAM
Retention time: 60

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics

REFERENCE
Fischer,V.; Baldeck,J.-P.; Tse,F.L.S. Pharmacokinetics and metabolism of the 5-hydroxytryptamine antagonist tropisetron after single oral doses in humans, *Drug Metab.Dispos.*, **1992**, *20*, 603–607.

Trospium chloride



Molecular formula: $C_{25}H_{30}ClNO_3$
Molecular weight: 427.97
CAS Registry No.: 10405-02-4
Merck Index: 9918

SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. 5 mL Plasma + 1 mL 1 M NaOH, heat at 140-145° for 105 min, cool to 40°, add 200 μL 25% HCl, mix, centrifuge at 5000 g for 10 min, heat at 120° for 10 min, cool, centrifuge. Remove a 5 mL aliquot and add it to 500 μL reagent 1, mix. Remove a 5 mL aliquot and add it to 4.8 mL chloroform, shake vigorously for 3 min, centrifuge at 6000 g for 30 min. Remove a 4 mL aliquot of the organic phase and add it to 2.3 mL 100 mM HCl, shake vigorously, centrifuge at 5000 g for 10 min. Remove a 2 mL aliquot of the aqueous layer and add it to 2 mL MeOH, evaporate to dryness under reduced pressure, add MeOH, evaporate to dryness under a stream of nitrogen at 80-90°, repeat process several times with 10-11 mL MeOH total, add 200 μL 10 mg/mL benoxaprofen chloride in dry MeCN, heat at 140-145° for 30 min, evaporate to dryness under reduced pressure, add 1 mL ethyl acetate, add 1.2 mL water, shake mechanically for 5 min, centrifuge at 5000 g for 10 min, discard the organic layer, wash the aqueous phase again with 1 mL ethyl acetate. Evaporate the aqueous phase to dryness under reduced pressure, reconstitute with 100 μL MeCN:water 31:69, inject a 20 μL aliquot. Urine. 5 mL Urine + 1 mL 1 M NaOH, heat at 140-145° for 90 min, cool, add 200 μL 25% HCl, add 600 μL reagent 2, shake, centrifuge at 5000 g for 10 min. Remove a 5 mL aliquot and add it to 4.8 mL chloroform, shake vigorously for 3 min, centrifuge at 6000 g for 30 min. Remove a 4 mL aliquot of the organic phase and add it to 2.3 mL 100 mM HCl, shake vigorously, centrifuge at 5000 g for 10 min. Remove a 2 mL aliquot of the aqueous layer and add it to 2 mL MeOH, evaporate to dryness under reduced pressure, add MeOH, evaporate to dryness under a stream of nitrogen at 80-90°, repeat process several times with 10-11 mL MeOH total, add 200 μL 10 mg/mL benoxaprofen chloride in dry MeCN, heat at 140-145° for 30 min, evaporate to dryness under reduced pressure, add 1 mL ethyl acetate, add 1.2 mL water, shake mechanically for 5 min, centrifuge at 5000 g for 10 min, discard the organic layer, wash the aqueous phase again with 1 mL ethyl acetate. Evaporate the aqueous phase to dryness under reduced pressure, reconstitute with 100 μL MeCN:water 40:60, inject a 20 μL aliquot. (Trospium chloride is hydrolyzed to nortropane-8-spiro-1'-pyrrolidinium chloride (which is also a metabolite) and this compound is derivatized with benoxaprofen chloride. Reagent 1 was a mixture of 98.7 mg dipicrylamine containing 50% water, 10 mL 100 mM NaOH, and 600 mg anhydrous sodium carbonate. Reagent 2 was a mixture of 32.9 mg dipicrylamine containing 50% water and 10 mL 100 mM NaOH. Prepare dipicrylamine as follows (Caution! Dipicrylamine is potentially explosive and highly toxic, store moistened with 50% water!). Add 50 g 2,4-dinitrodiphenylamine to 420 g nitric acid (36° Bé., 52%, d 1.33) heated to 62° over 2 h, heat at 62-90° for another 3 h, cool, filter, wash the product until it is free of acid, dry to obtain 2,2',4,4'-tetranitrodiphenylamine as a yellow solid (mp 187.4°). Add 50 g tetranitrodiphenylamine over 1 h to 500 g of a mixture of equal parts 92% sulfuric acid and 93% nitric acid at room temperature, after 4.5 h add to a large volume of ice water, filter, recrystallize the product from acetone to obtain dipicrylamine (2,2',4,4',6,6'-hexanitrodiphenylamine) as yellow crystals (mp 242.9°) (J.Am.Chem.Soc. 1919, 41, 1013). Prepare benoxaprofen chloride as follows. Dissolve 600 mg benoxaprofen in 50 mL dry toluene, slowly add 5 mL thionyl chloride (freshly distilled from linseed oil), reflux for 30 min, evaporate to dryness, recrystallize from dichloromethane (if necessary) to give benoxaprofen chloride (mp 91.5°) (J. Chromatogr. 1984, 310, 167).)

HPLC VARIABLES

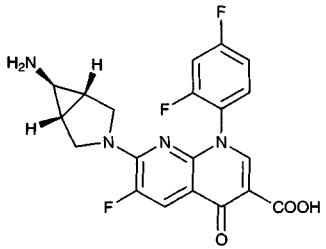
Column: 125 × 4.6 5 μm Nucleosil C8 (plasma) or 125 × 4.6 5 μm LiChrosorb RP-8 (urine)
Mobile phase: MeCN:water 31:69 (plasma) or 40:60 (urine) containing 80 mM NaCl, 31 mM choline chloride, and 10 mL/L 1 M HCl
Column temperature: 55 (plasma), 50 (urine)
Flow rate: 2
Injection volume: 20
Detector: F ex 313 em 370

CHROMATOGRAM
Retention time: 7.5 (plasma), 6 (urine)
Limit of quantitation: 0.5-1 ng/mL (plasma), 3 ng/mL (urine)

KEY WORDS
derivatization; plasma; pharmacokinetics

REFERENCE
Schladitz-Keil,G.; Spahn,H.; Mutschler,E. Fluorimetric determination of the quaternary compound trospium and its metabolite in biological material after derivatization with benoxaprofen chloride, *J.Chromatogr.*, **1985**, *345*, 99–110.

Trovafloxacin



Molecular formula: $C_{20}H_{15}F_3N_4O_3$
Molecular weight: 416.36
CAS Registry No.: 147059-72-1, 146961-34-4 (HCl), 147059-75-4 (monomethanesulfonate)
Merck Index: 9919

SAMPLE
Matrix: plasma
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 22:78 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1
Detector: UV 274

CHROMATOGRAM
Retention time: 8.57
Internal standard: fleroxacin (3.93)

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

Trypsin

Molecular weight: 24000
CAS Registry No.: 9002-07-7
Merck Index: 9926

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 μBondapak CN
Mobile phase: Gradient. MeCN containing 0.07% trifluoroacetic acid:0.1% trifluoroacetic acid in water from 0:100 to 60:40 over 30 min
Flow rate: 2
Detector: UV 206

CHROMATOGRAM
Retention time: 23.8

KEY WORDS
partial separation of α and β forms

REFERENCE
Titani,K.; Sasagawa,T.; Resing,K.; Walsh,K.A. A simple and rapid purification of commercial trypsin and chymotrypsin by reverse-phase high-performance liquid chromatography, *Anal.Biochem.*, **1982**, *123*, 408–412.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 500 μg/mL solution in 1 mM HCl, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 6 Asahipak GS-520-AHA-ABA (Prepare by suspending 10 g Asahipak-GS gel (Asahi Chemical Industry) in water, sonicate for 5 min, wash with 200 mL water, wash with 200 mL dioxane, suspend in 100 mL dioxane, add 3.24 g 1,1'-carbonyldiimidazole, stir gently for 15 min at room temperature, wash with 200 mL dioxane, suspend in 200 mL 1 M sodium bicarbonate containing 1 M 6-aminohexanoic acid, shake at 4° for 25 h, wash with 200 mL water, wash with 100 mL 1 M NaCl, wash with 200 mL water. Suspend 2 g of the gel in 15 mL 200 mM pH 4.752-(morpholino)ethanesulfonic acid/NaOH buffer, add 288 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide monohydrochloride, stir gently for 30 min, add 28.3 mg p-aminobenzamide monohydrochloride, adjust pH three times to 4.75 with 1 M HCl or 1 M NaOH at 30 min intervals, shake gently at room temperature for 24 h, wash with 150 mL water, wash with 100 mL 50 mM NaOH containing 1 M NaCl, wash with 100 mL 50 mM HCl containing 1 M NaCl, wash with water until washings are neutral. Caution! Dioxane is a carcinogen. It may be possible to use acetone instead.)
Mobile phase: Gradient. Buffer A, after 15 min buffer B. Buffer A was 50 mM pH 7.4 sodium phosphate buffer containing 100 mM NaCl. Buffer B was 50 mM pH 7.4 sodium phosphate buffer containing 100 mM NaCl and 20 mM pH 7.4 6-aminohexanoic acid.
Flow rate: 1
Injection volume: 20
Detector: F ex 285 em 340

CHROMATOGRAM
Retention time: 25

KEY WORDS
cow

REFERENCE
Ito,N.; Noguchi,K.; Shimura,K.; Kasai,K.-I. High-performance affinity chromatography of trypsins on Asahipak GS-gel coupled with *p*-aminobenzamidine, *J.Chromatogr.*, **1985**, *333*, 107–114.

SAMPLE
Matrix: solutions

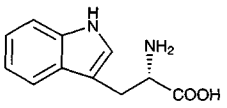
HPLC VARIABLES
Column: 150 × 4.1 10 μm PRP-3 (Hamilton)
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in 50 mM NaOH. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 40:60 over 30 min.
Flow rate: 2
Detector: UV 220

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: cytochrome C, insulin, lysozyme, myoglobin, ribonuclease A

REFERENCE
Rainin Catalog 1991-2, p. 3.33, p. 3.33.

Tryptophan



Molecular formula: $C_{11}H_{12}N_2O_2$
Molecular weight: 204.23
CAS Registry No.: 73-22-3
Merck Index: 9929

SAMPLE
Matrix: solutions

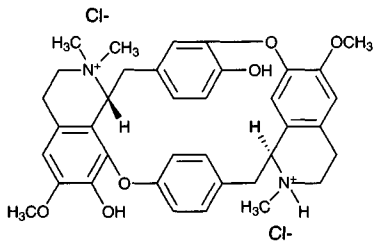
HPLC VARIABLES
Column: C18
Mobile phase: MeOH:MeCN:2.5 nM pH 3.0 ammonium dihydrogen phosphate buffer 17.5:17.5:65
Detector: UV 278

CHROMATOGRAM
Retention time: 4.2
Internal standard: tryptophan

KEY WORDS
tryptophan is IS

REFERENCE
Duggirala,S.M.; Mitra,A.K. Intravitreal pharmacokinetics of anti CMV agents ganciclovir and cidofovir -a comparison (Abstract 1119), *Pharm.Res.*, **1997**, *14*, S39.

Tubocurarine chloride



Molecular formula: $C_{37}H_{42}Cl_2N_2O_6$
Molecular weight: 681.66
CAS Registry No.: 57-94-3, 6989-98-6 (pentahydrate)
Merck Index: 9939

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C18 SPE cartridge with 2 column volumes of THF, 2 volumes of MeOH, and 2 volumes of water. 1 mL Plasma + 100 µL 10 µg/mL metocurine iodide in 10 mM HCl, add to the SPE cartridge, wash with 2 volumes of water, elute with 250 µL mobile phase. Evaporate the eluate and reconstitute with 100 µL mobile phase, vortex, centrifuge at 12800 g for 5 min, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm CN Guard-Pak (Waters)
Column: 10 μm Radial-Pak CN (Waters)
Mobile phase: MeCN:MeOH:water:1 M pH 2.5 dibutylamine phosphate 40:10:10:1
Flow rate: 2.4
Detector: UV 204

CHROMATOGRAM
Retention time: 5.9
Internal standard: metocurine iodide (13.2)
Limit of quantitation: 25 ng/mL

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Avram,M.J.; Shanks,C.A. Determination of D-tubocurarine chloride or metocurine iodide in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1984**, *306*, 398–404.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Serum + 10 μL water + 50 μL 10% sodium tungstate:335 mM sulfuric acid 50:50, vortex for 15 s, centrifuge at 12800 g for 2 min, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 50 mm long C18
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:7.5 mM tetrabutylammonium hydrogen sulfate 10:90
Flow rate: 1.8
Injection volume: 30
Detector: UV 229

CHROMATOGRAM
Retention time: 10.0
Internal standard: d-tubocurarine chloride

OTHER SUBSTANCES
Extracted: gallamine
Noninterfering: barbiturates, alcuronium, metocurine, neostigmine, edrophonium

KEY WORDS
serum; rat; tubocurarine is IS

REFERENCE
Ramzan,I.M. Determination of the neuromuscular blocking drug gallamine in rat serum using high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *417*, 428–433.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 250 μL picric acid (1:50 dilution of saturated picric acid solution) + 250 μL metocurine solution + 250 μL water + 2.5 mL dichloromethane:isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 μL MeCN: water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 μPorasil
Mobile phase: MeCN:2 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100

Detector: UV 210

CHROMATOGRAM
Retention time: 3.7
Internal standard: metocurine (5.3)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Also analyzed: atracurium, alcuronium

KEY WORDS
plasma

REFERENCE
Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE
Matrix: blood
Sample preparation: 10 mL Blood + 1 mL 1 M pH 2.5 KH_2PO_4 + 1 mL 3% perchloric acid + 12 mL dichloromethane, rotate for 20 min, centrifuge at 1520 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Guard column: 25 mm long CN guard column
Column: 250 × 4.6 Spherisorb S5 CN
Mobile phase: MeCN:100 mM pH 5 phosphate 50:50
Column temperature: 40
Flow rate: 1.5
Detector: UV 214 or E, BAS LC-4B, LC-17A thin-layer flow cell, working glassy carbon electrode (W1) +0.65 V, quantitating glassy carbon electrode (W2) +1.05 V, Ag/AgCl reference electrode and auxiliary electrode

CHROMATOGRAM
Internal standard: d-tubocurarine

OTHER SUBSTANCES
Extracted: vecuronium
Noninterfering: atropine, apresoline, haloperidol, fentanyl, labetalol, thiopental, atracurium, diazepam

KEY WORDS
tubocurarine is IS

REFERENCE
Hu,O.Y.; Chou,C.H.; Ho,W.; Ho,S.T. Determination of vecuronium in blood by HPLC with UV and electrochemical detection: a pilot study in man, *Proc.Natl.Sci.Counc.Repub.China.[B].*, **1991**, *15*, 186–190.

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut CBA cation-exchange SPE cartridge with 3 mL MeOH, 3 mL water, 1 mL 50 mM pH 9.0 borate. Add 250 μL plasma to SPE cartridge, wash with 3 mL water, wash with 1 mL 50 mM pH 3.0 NaH_2PO_4, wash with 1 mL water, wash with two 500 μL portions of MeOH, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 200 μL MeOH:MeCN:water 30:15:55 adjusted to pH 3.4 with 1 M phosphoric acid, inject a 70 μL aliquot. (Acidified MeOH was 833 μL HCl in 100 mL MeOH.)

HPLC VARIABLES
Column: 100 × 4.9 5 μm octyl Spherisorb
Mobile phase: MeOH:MeCN:buffer 30:15:55 adjusted to pH 3.4 with 1 M phosphoric acid (Buffer was 10 mM sodium octanesulfonate and 1.5 mM dibutylamine.)
Flow rate: 2.5
Injection volume: 70
Detector: UV 272

CHROMATOGRAM
Retention time: 5
Internal standard: D-tubocurarine

OTHER SUBSTANCES
Extracted: bretylium

KEY WORDS
plasma; SPE; human; pig; tubocurarine is IS

REFERENCE
Théorêt,Y.; Varin,F. Simple, rapid and selective method using high-performance liquid chromatography for the determination of bretylium in plasma, *J.Chromatogr.*, **1992**, *575*, 162–166.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 100 mg Bond Elut SPE cartridge with 2 mL water and 1 mL MeOH. Add 1 mL plasma or 10 mL urine to the SPE cartridge, wash with 2 mL water, wash with 1 mL MeCN:water 50:50, elute with 300 μL MeCN:buffer 50:50, inject an aliquot. (Buffer was 12 g NaH_2PO_4 and 1.2 mL concentrated phosphoric acid in 100 mL water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Waters)
Mobile phase: MeOH:buffer 80:20 (Buffer was 1.44 g sodium lauryl sulfate and 2.5 mL glacial acetic acid in 1 L water.)
Flow rate: 1.4
Detector: UV 280

CHROMATOGRAM
Retention time: 7.2
Internal standard: d-tubocurarine

OTHER SUBSTANCES
Extracted: alcuronium

KEY WORDS
SPE; tubocurarine is IS; plasma

REFERENCE
deBros,F.; Okutani,R.; Inada,E.; Lawrence,K. Determination of alcuronium in plasma and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 449–454.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg silica gel SPE cartridge (Analytichem) with 2 mL ethyl acetate, 2 mL MeOH, and 2 mL water. Lyophilize 40 mL urine, reconstitute with 10 mL 100 mM pH 5.0 acetate buffer, centrifuge at 5000 g for 15 min. If desired, incubate a 3 mL aliquot with 10 μL of a solution containing 500 U/μL glucuronidase and 26.4 U/μL sulfatase (Sigma), heat at 37° overnight, freeze. Lyophilize 3 mL aliquots of hydrolyzed or unhydrolyzed solutions, reconstitute with 2 mL water, add 10 μL 2.8 mg/mL D-chondocurarine in water. Remove a 200 μL aliquot and add it to the SPE cartridge, wash with 2 mL water, wash with 500 μL MeOH, elute with two 1 mL portions of mobile phase, inject an aliquot of the eluate.

Alternatively add 10 μL 60 ng/mL D-chondocurarine in water to 1 mL urine, add this mixture to the SPE cartridge and proceed as above.

HPLC VARIABLES

Guard column: 20 × 4.6 10 μm C18 (Rainin)
Column: 150 × 4.6 5 μm ODS-IP (Beckman)
Mobile phase: MeOH:water:dibutylamine phosphate solution 19.8:79.1:0.1, adjust pH to 2.5 with 1 M NaOH (Prepare dibutylamine phosphate solution by cautiously adding 32 g dibutylamine to 250 mL concentrated orthophosphoric acid.)
Flow rate: 1.5
Injection volume: 200
Detector: UV 204

CHROMATOGRAM

Retention time: 7.5
Internal standard: D-chondocurarine (10)
Limit of detection: 15 ng/mL

KEY WORDS

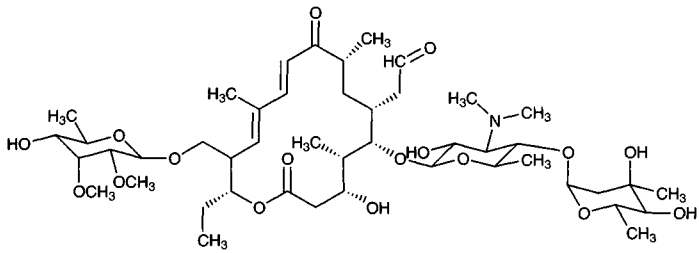
SPE

REFERENCE

Annan,R.S.; Kim,C.; Martyn,J. Measurement of D-tubocurarine chloride in human urine using solid-phase extraction and reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1990**, *526*, 228–234.

Tylosin

Molecular formula: $C_{46}H_{77}NO_{17}$
Molecular weight: 916.11
CAS Registry No.: 1401-69-0
Merck Index: 9963



SAMPLE

Matrix: blood, tissue
Sample preparation: Blend 25 g tissue with 3 volumes of water (muscle) or 200 mM pH 2.2 phosphate buffer (liver, kidney). Add 32 (tissue) or 30 (serum) mL MeCN slowly with vigorous swirling to 8 mL tissue homogenate or 10 mL serum, let stand for 1 min, decant through a glass wool plug. 20 mL Filtrate + 20 mL water + 30 mL dichloromethane, shake vigorously, repeat extraction. Combine the organic layers and evaporate them to near dryness under reduced pressure at 40-50°, reconstitute the residue in two 3 mL portions of MeOH and transfer to a small tube. Evaporate to dryness under reduced pressure, reconstitute with 1 mL MeCN and 1 drop of water, add 3 mL petroleum ether (bp 30-60°), vortex for 10 s, discard petroleum ether layer, repeat wash, remove traces of petroleum ether under reduced pressure, adjust volume to 0.2-1 mL with MeCN, inject an aliquot.

HPLC VARIABLES

Column: 300 × 4.6 10 μm Micropak MCH-10-N-Cap C18
Mobile phase: MeCN:MeOH:5 mM $(NH_4)H_2PO_4$ 60:30:10, 65:30:5, or 70:25:5 or MeCN:MeOH:4 mM $(NH_4)H_2PO_4$ 72:20:8 (tissue) or MeCN:MeOH:2 mM $(NH_4)H_2PO_4$ 75:20:5 or 75:18:7 (serum)
Flow rate: 1.5-2
Injection volume: 200
Detector: UV 280

CHROMATOGRAM

Limit of detection: <100 ppb

KEY WORDS
muscle; liver; kidney; pig; serum

REFERENCE
Moats,W.A.; Harris,E.W.; Steele,N.C. Comparison of liquid chromatographic and bioassay procedures for determining depletion of intramuscularly injected tylosin, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 413–416.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 1 mg/mL solution in water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 8 μm RoSil C8 (Bio-Rad)
Mobile phase: MeCN:water:200 mM tetrabutylammonium hydrogen sulfate:200000 mM phosphoric acid 20:67:8:5
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 0.05% (of tylosin A)

OTHER SUBSTANCES
Simultaneous: impurities, demycinosyltylosin

REFERENCE
Roets,E.; Beirinckx,P.; Quintens,I.; Hoogmartens,J. Quantitative analysis of tylosin by column liquid chromatography, *J.Chromatogr.*, **1993**, *630*, 159–166.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 1 mg/mL solution in 50 mM pH 7.0 potassium phosphate buffer, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 8 μm 1000 Å PLRP-S (Polymer Laboratories)
Mobile phase: THF:water:200 mM pH 9.0 potassium phosphate buffer 20:75:5
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 37

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
protect from light

REFERENCE
Paesen,J.; Crommen,J.; de Beer,J.; Shaohong,J.; Porqueras,E.; Van Overbeke,A.; Violon,C.; Hoogmartens,J. Collaborative study of the analysis of tylosin by liquid chromatography on wide-pore poly(styrene-divinylbenzene), *J.Liq.Chromatogr.*, **1995**, *18*, 1195–1205.

SAMPLE
Matrix: bulk

Sample preparation: Prepare a 1 mg/mL solution in 40 mM K_2HPO_4, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 8 μm 1000 Å PLRP-S poly(styrene-divinylbenzene) (Polymer Labs)
Mobile phase: THF:water:200 mM pH 9.0 potassium phosphate buffer 20:75:5
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 29 (tylosin A)
Limit of detection: 0.06%

OTHER SUBSTANCES

Simultaneous: impurities

REFERENCE

Paesen,J.; Claeys,P.; Cypers,W.; Roets,E.; Hoogmartens,J. Liquid chromatography of tylosin A and related substances on poly(styrene-divinylbenzene), *J.Chromatogr.A*, **1995**, *699*, 93–97.

SAMPLE

Matrix: feed
Sample preparation: Mix 25 g feed with 100 mL MeOH:100 mM pH 4.0 phosphate buffer 50:50, shake for 1 h, centrifuge at 2500 rpm for 5 min, gently add several mL of the supernatant to the top of a dry column containing 3 mL acidic alumina (J.T. Baker), discard the first 2 mL and collect the next 2-4 mL, inject a 20 μL aliquot. (Prepare 100 mM pH 4.0 phosphate buffer as follows. Dissolve 16.73 g anhydrous K_2HPO_4 and 523 mg anhydrous KH_2PO_4 in water and dilute to 1L with water.)

HPLC VARIABLES

Guard column: 10 × 4.6 5 μm Econosphere C8
Column: 150 × 4.6 5 μm Econosphere C8
Mobile phase: Gradient. MeOH:buffer from 40:60 to 60:40 over 12 min, maintain at 60:40 until no other peaks elute (ca. 2 min), re-equilibrate at 40:60 for 5 min. (Prepare buffer as follows. Dissolve 5 g tetramethylammonium chloride in water, add 5 mL glacial acetic acid, make up to 1 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 7.4 (tylosin B), 9.3 (tylosin-urea adduct), 10.7 (tylosin A),
Limit of quantitation: 4 μg/g

OTHER SUBSTANCES

Noninterfering: monensin, sulfamethazine

KEY WORDS

feed

REFERENCE

Houglum,J.E.; Tasler,M.K. Liquid chromatographic assay of tylosin in animal feeds, *J.AOAC Int.*, **1996**, *79*, 369–374.

SAMPLE

Matrix: feed
Sample preparation: 10 g Ground feed + 100 mL methanolic NaCl, sonicate for 1 h, cool to room temperature, filter through a glass wool pad, wash with three 20 mL portions of methanolic NaCl, make up filtrate to 200 mL with methanolic NaCl, mix. Add a 25 mL aliquot of the filtrate to 10 mL 550 mg/mL KI in water, mix, add 50 mL chloroform, shake vigorously for

10 s, repeat extraction. Combine the chloroform layers and add them to 25 mL 100 mM NaOH, shake vigorously for 10 s. Remove the organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 10 mL mobile phase, inject a 25 μL aliquot. (Prepare methanolic NaCl by mixing 1 L 100 g/L NaCl in water and 1 L MeOH, prepare fresh daily.)

HPLC VARIABLES
Guard column: 400 mg Corasil II
Column: 250 × 4.6 Zorbax Sil
Mobile phase: MeCN:acetic acid:water:diethylamine 94:2.5:2.5:1
Flow rate: 1.6
Injection volume: 25
Detector: UV 313

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: pyrantel
Noninterfering: carbadox, lincomycin

KEY WORDS
protect from light

REFERENCE
Goras,J.T. High performance liquid chromatographic method for pyrantel tartrate in swine feeds and supplements, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 1291–1296.

SAMPLE
Matrix: formulations, premix
Sample preparation: Premixes, powders. Shake with MeCN:water 50:50 so as to produce a 0.02% solution, inject a 20 μL aliquot. Tablets. Powder tablets, weigh out amount equivalent to 200 mg tylosin, add 50 mL MeOH, shake, filter. Remove a 5 mL aliquot of the filtrate and make up to 100 mL with MeCN:water 50:50, inject a 20 μL aliquot. Injections. Dilute 1 mL injection to 250 mL with MeCN:water 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeCN:850 mM sodium perchlorate 40:60, adjusted to pH 2.5 with 1 M HCl
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 14 (tylosin A)

OTHER SUBSTANCES
Simultaneous: impurities, desmycosin, desmycinosyl tylosin, macrocin, relomycin

KEY WORDS
powders; tablets; injections

REFERENCE
Fish,B.J.; Carr,G.P.R. Pharmacopoeial procedure for the determination of tylosin factors by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *353*, 39–50.

SAMPLE
Matrix: reaction mixtures
Sample preparation: Adjust pH of 500 μL enzyme reaction mixture in 100 mM pH 7.8 potassium phosphate buffer to 2.6 with trichloroacetic acid, add 5.6 nmoles relomycin, adjust pH to 12.9 with NaOH, add 1 mL ethyl acetate, shake at 60° for 10 min, centrifuge at 300 g for 5 min, inject a 125 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 4.6 6 μm Zorbax C8
Mobile phase: MeCN:THF:buffer 20:15:65 (Buffer was 1% acetic acid containing 0.025% 1-pentanesulfonic acid.)
Column temperature: 55
Flow rate: 2
Injection volume: 125
Detector: UV 285

CHROMATOGRAM
Retention time: 6.5
Internal standard: relomycin (5)
Limit of quantitation: 200 pmoles

OTHER SUBSTANCES
Simultaneous: macrocin

REFERENCE
Yeh,W.K.; Bauer,N.J.; Dotzlaf,J.E. High-performance liquid chromatographic assay for S-adenosyl-L-methionine:macrocin O-methyltransferase, *J.Chromatogr.*, **1984**, *288*, 157–165.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 90:10, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18 end-capped
Mobile phase: MeCN:MeOH:4 mM $(NH_4)H_2PO_4$ 70:25:5
Flow rate: 1
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 10

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 8 μm 1000 Å PLRP-S (Polymer Laboratories)
Mobile phase: THF:200 mM pH 9.0 potassium phosphate buffer:water 20:5:75
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 28 (tylosin A)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Paesen,J.; Cypers,W.; Pauwels,K.; Roets,E.; Hoogmartens,J. Study of he stability of tylosin A in aqueous solutions, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1153–1159.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm C18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
Flow rate: 0.5
Injection volume: 200
Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 562.3-916.5 in NCI and 916.5-773.4 in PCI

CHROMATOGRAM
Retention time: 6.1
Limit of detection: 50 μg/kg

OTHER SUBSTANCES
Extracted: erythromycin, josamycin, spiramycin, tilmicosin

KEY WORDS
muscle; cow; SPE

REFERENCE
Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut SCX SPE cartridge (Varian) with 5 mL MeOH and 10 mL 100 mM pH 4.4 KH_2PO_4 buffer. Homogenize 5 g tissue with 100 mL MeOH: 0.3% metaphosphoric acid 30:70 at high speed for 2 min, filter through 2 mm Hyflo Super-Cel coated on a suction funnel (when filtering liver or kidney add several grams of Hyflo Super-Cel to the homogenized solution before filtration). Evaporate the filtrate to ca. 20 mL under reduced pressure at 45°, add to the SPE cartridge, wash with 10 mL distilled water and 5 mL 100 mM pH 8.9 K_2HPO_4 buffer, elute with 10 mL MeOH, evaporate the eluate to dryness under reduced pressure at 45°, dissolve the residue in 1 mL MeCN:50 mM pH 4.5 NaH_2PO_4 buffer 30:70, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Puresil 5C18 (Waters)
Mobile phase: Gradient. A:B from 60:40 to 0:100 over 16 min. A was buffer. B was MeCN:buffer 40:60 (Buffer was 2.5 g KH_2PO_4 dihydrate and 0.65 mL 85% phosphoric acid dissolved in 1 L distilled water, pH 2.5.)
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 232 for 9 min, UV 287 for 2 min, UV 232 for 4 min

CHROMATOGRAM
Retention time: 10.2
Limit of detection: 50 ng/g

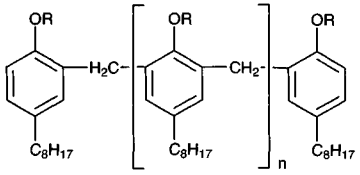
OTHER SUBSTANCES
Extracted: josamycin, leucomycin (kitasamycin), mirosamicin, spiramycin

KEY WORDS
meat; SPE

REFERENCE
Horie,M.; Saito,K.; Ishii,R.; Yoshida,T.; Haramaki,Y.; Nakazawa,H. Simultaneous determination of five macrolide antibiotics in meat by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 295–302.

Tyloxapol

CAS Registry No.: 25301-02-4
Merck Index: 9964



R = $CH_2CH_2O(CH_2CH_2O)_mCH_2CH_2OH$;
m is 6 to 8; n is not more than 5

SAMPLE
Matrix: formulations
Sample preparation: Condition a 1 mL Supelcoclean cyano SPE cartridge with 2 mL MeCN and 2 mL water. Add 4 mL formulation to the SPE cartridge, wash with 2 mL MeCN:buffer 30:70, elute with 5 mL mobile phase, make up eluate to 10 mL with water, inject a 100 µL aliquot. (Buffer was 6 mL concentrated phosphoric acid in 1950 mL water, adjust pH to 5.0 with 50% NaOH, make up to 2 L with water.).

HPLC VARIABLES
Column: 150 × 4.6 5 µm Ultrasphere cyano
Mobile phase: MeCN:buffer 60:40 (Buffer was 6 mL concentrated phosphoric acid in 1950 mL water, adjust pH to 5.0 with 50% NaOH, make up to 2 L with water.)
Flow rate: 2
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Also analyzed: benzalkonium C10-C18

KEY WORDS
ophthalmic solutions; eye; SPE

REFERENCE
Fan,T.Y.; Wall,G.M. Determination of benzalkonium chloride in opthalmic solutions containing tyloxapol by solid-phase extraction and reversed-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1993**, *82*, 1172–1174.

SAMPLE
Matrix: solutions

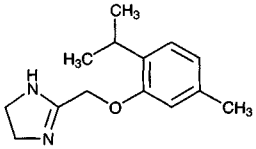
HPLC VARIABLES
Column: 250 × 4.6 diol (Supelco)

Mobile phase: Gradient. A was dichloromethane:MeOH 90:10. B was hexane. A:B from 10:90 to 90:10 over 18 min, return to initial conditions over 2 min.
Flow rate: 1
Detector: UV 280

REFERENCE

DeAngelis,R.L.; Kearney,M.F.; Barnes,E.R.; Shockcor,J.P.; Findlay,J.W.A. Balance/excretion of ^{3}H- and ^{14}C-tyloxapol in the male rabbit after intratracheal administration, *Xenobiotica*, **1995**, *25*, 521–530.

Tymazoline



Molecular formula: $C_{14}H_{20}N_2O$
Molecular weight: 232.33
CAS Registry No.: 24243-97-8
Merck Index: 9965

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 16.00

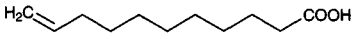
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Undecylenic acid



Molecular formula: $C_{11}H_{20}O_2$
Molecular weight: 184.28
CAS Registry No.: 112-38-9, 557-08-4 (Zn salt)
Merck Index: 9983

SAMPLE

Matrix: blood
Sample preparation: Perform all operations with the exclusion of light. Evaporate 240 μL derivatization solution into a vial, add 400 μL 50 mM pH 7.0 phosphate buffer, add 100 μL